A Comprehensive *In Silico* and *In Vitro* Immunogenicity Risk Assessment of Dalcinonacog Alfa Shows No Increased Risk Compared with Wild-Type FIX

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CATALYST BIOSCIENCES

Dalcinonacog alfa

Dalcinonacog alfa, a novel clinical stage SQ FIX product candidate differentiated from IV market leaders:

- Simpler, less painful, small dose
- SQ enhances pharmacokinetics
- Potential to maintain continuous protective levels
- Disruptive to all current intravenous products
- Especially well suited for children

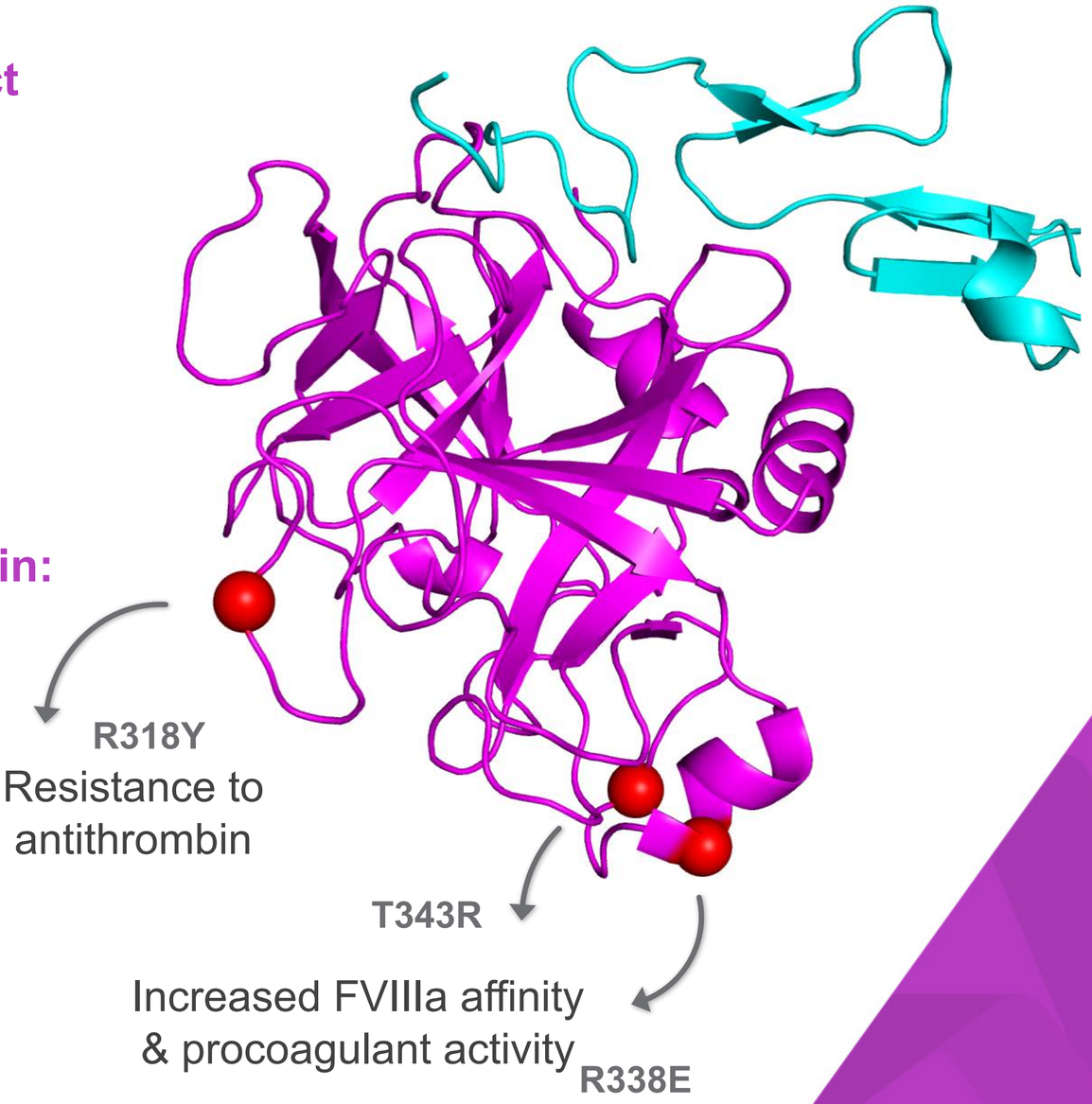
Three point mutations in two loops within the FIX protein:

- Catalytic activity increased
- Affinity for activated factor VIII increased
- Resistance to inhibition by antithrombin improved

Best-in-class high-potency recombinant FIX product

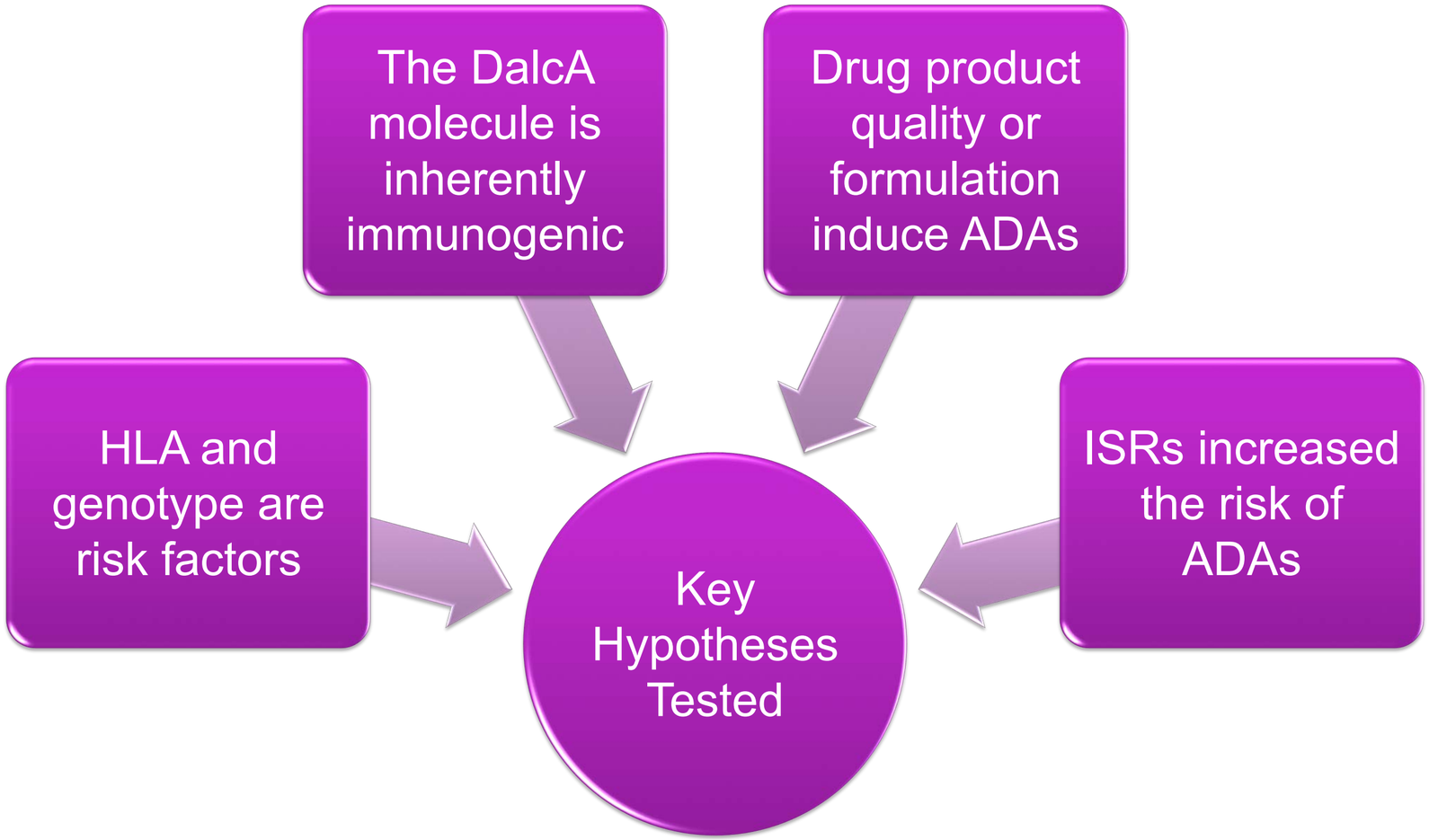
- 22-fold more potent than BeneFIX in man

Orphan Drug Designation in US & EU



Retrospective immunogenicity assessment

A comprehensive assessment of immunogenicity addressed several key hypotheses



"Considering our global and regional *in silico* analysis alongside whole protein and peptide *in vitro* experiments … we find the risk that wildtype FIX and therapeutic candidate DalcA will create or contribute to anti-therapeutic immune response to be minimal."

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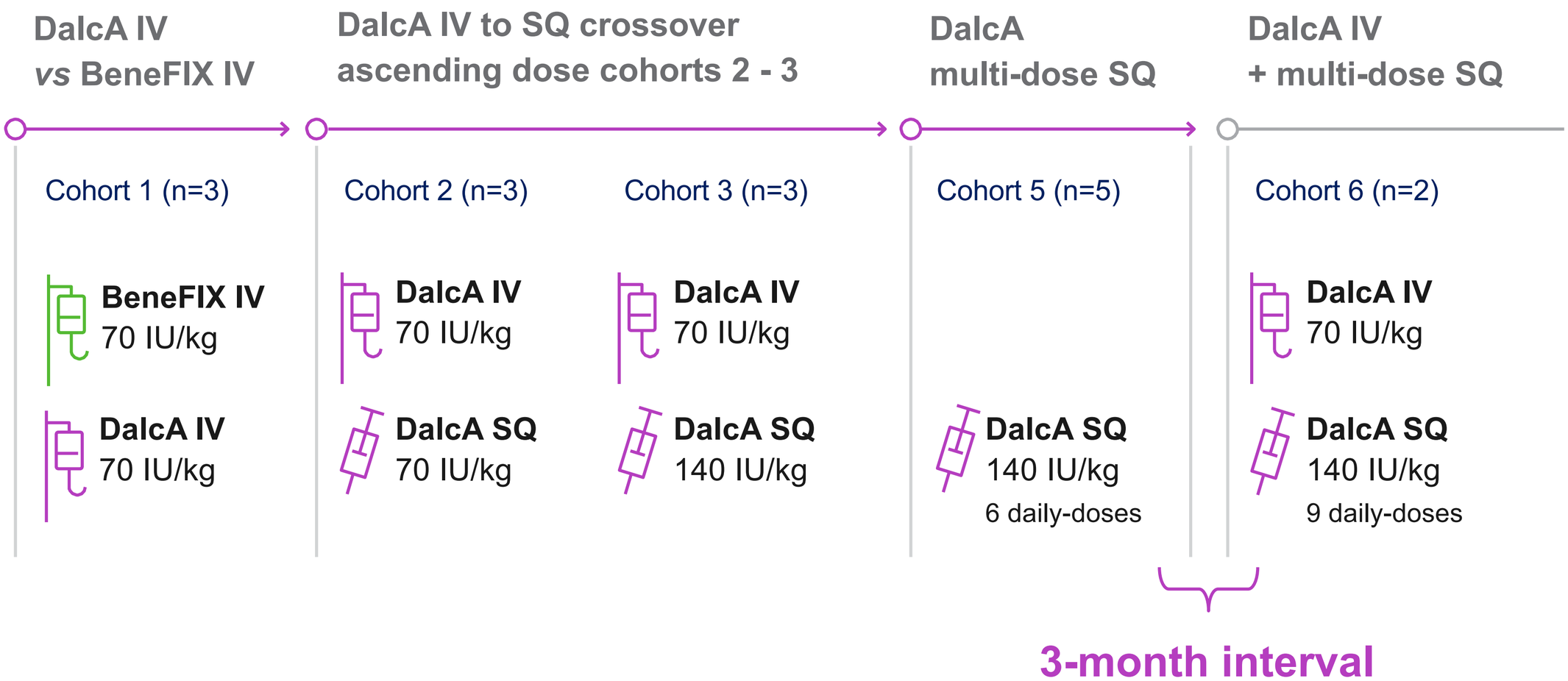
Dalcinonacog Phase 1/2 open label design

Nasdaq: CBIO

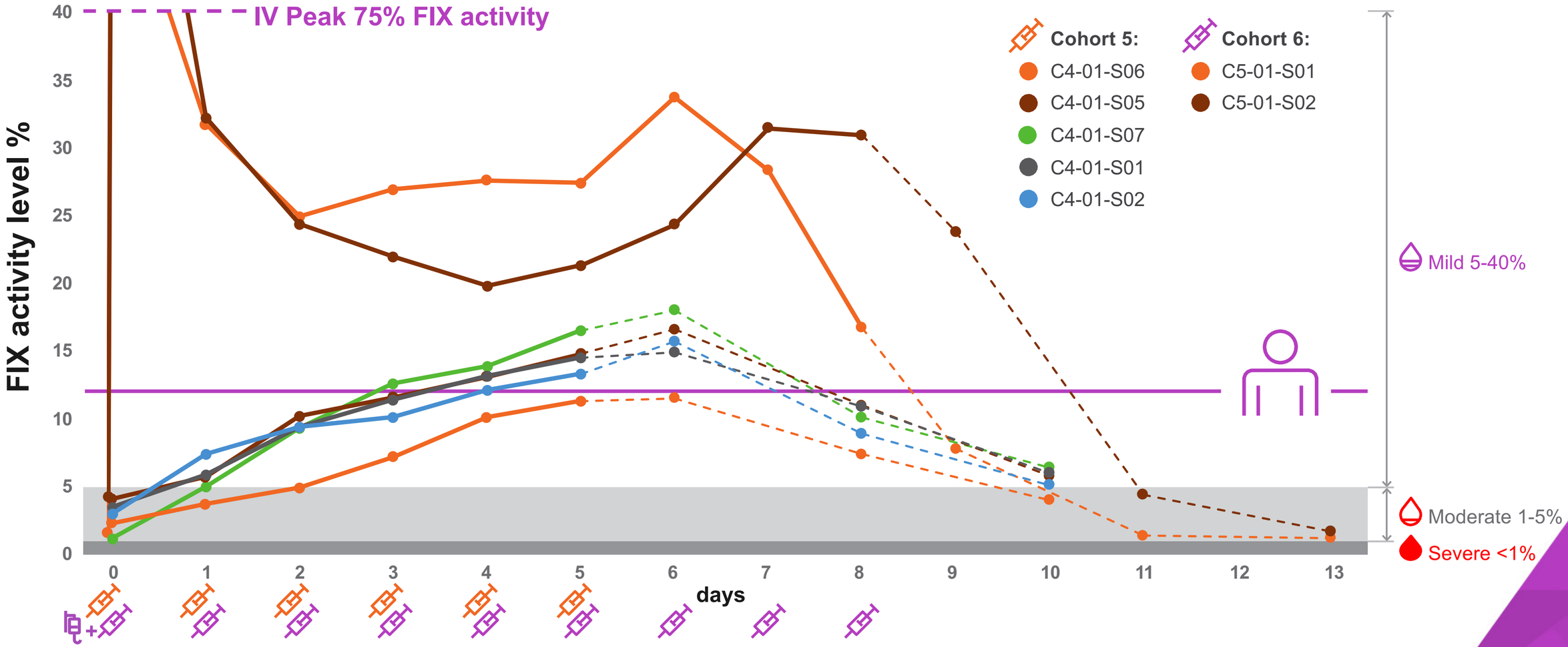
Subcutaneous treatment of hemophilia B



Phase 1/2: Cohort 5 & 6 FIX activity results

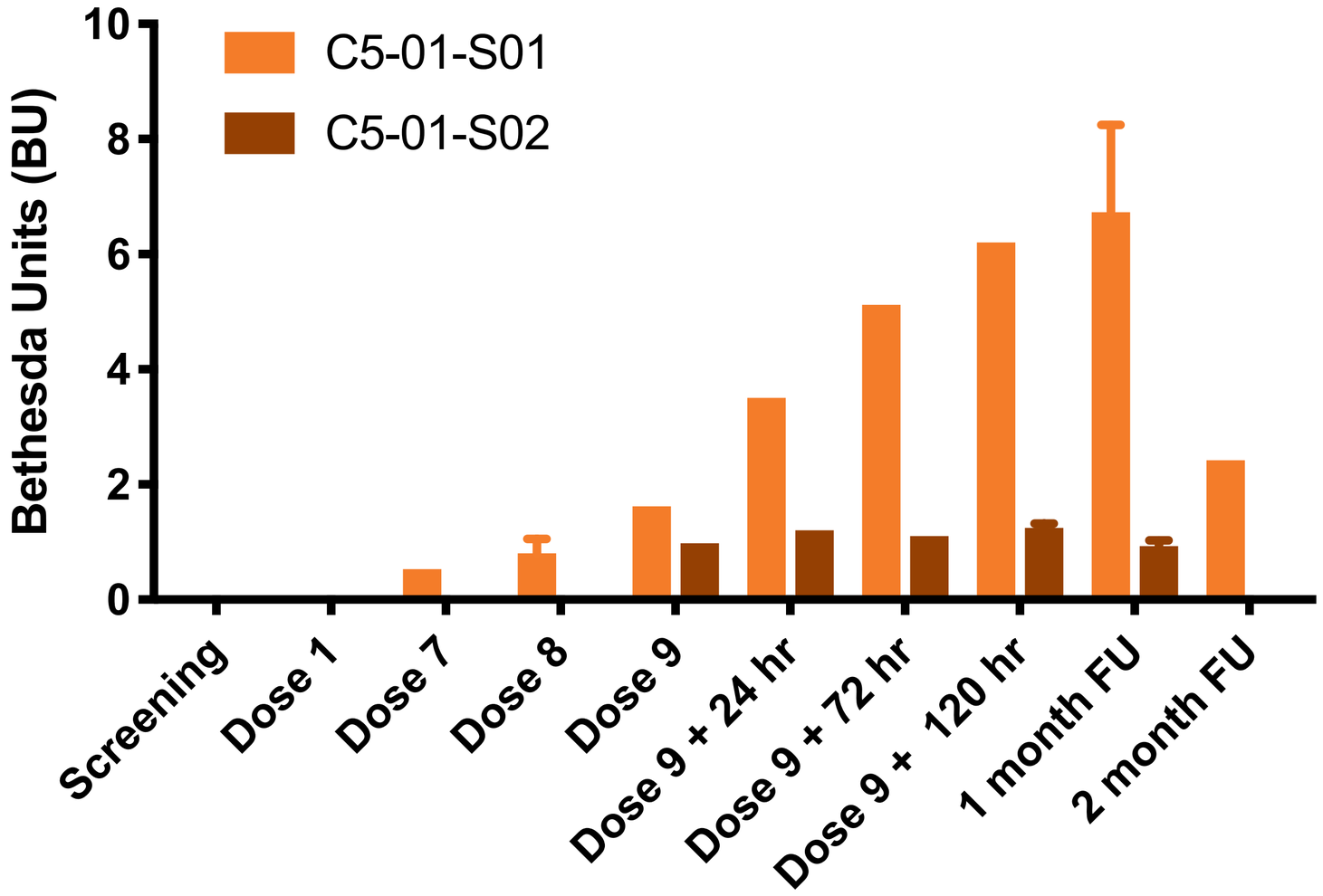
Nasdaq: CBIO

6/7 patients had trough levels >12%, sufficient to protect against spontaneous joint bleeds



Phase 1/2: Cohort 6 FIX nAb development timeline

Time course of neutralizing antibody development after prior exposure in Cohort 5



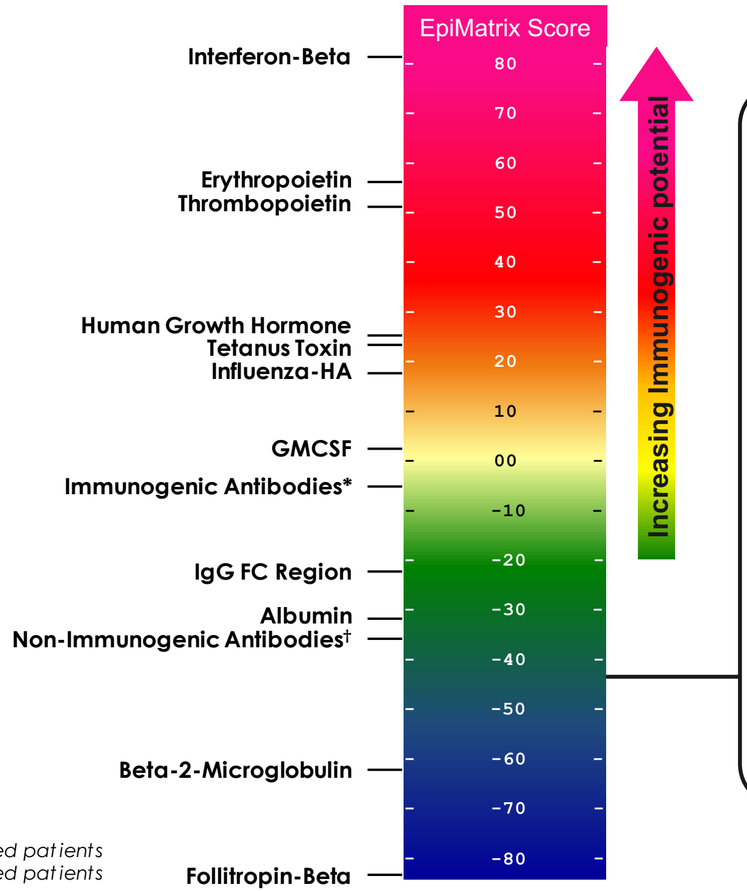
HLA and genotype of the subjects who developed nAbs

Nasdaq: CBIO

Subject ID	DRB1		DQB1		DPB1		Genotype	Phenotype
C5-01-S01	03:01	04:01	02:01	03:01	02:01	02:01	128G>A	Arg43Gln: propeptide
C5-01-S02	01:01	13:01	05:01	06:01	02:01	04:01	128G>A	Arg43Gln: propeptide

- The two subjects in cohort 6 that developed the nAbs are cousins and have the same genotype
  - Genotype is an Arg to Gln mutation at amino acid -4 (defective propeptide cleavage site)
- Only common HLA type is DPB1 02:01

In silico immunogenicity assessment shows low risk



*ADA in > 5% exposed patients
†ADA in < 5% exposed patients

Molecule	EpiMatrix Score	Average Response Intensity	Percent of Donors Responding
BeneFIX Lot A	-41.65	0.76% OB	10%
DalcA Phase 1/2	-42.54	0.97% OB	15%
BeneFIX Lot B	-41.65	2.84% OB	2%
DalcA Phase 2b	-42.54	0.92% OB	4%

OB: Over Background

+ EpiMatrix Protein Scores reflect an excess or shortfall in putative T-cell epitope content relative to random expectation (predicted using the EpiMatrix system)
+ *In vitro* DC-T cell assays demonstrated minimal response above unstimulated control background for both sequences, confirming *in silico* prediction of low immunogenicity

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DalcA shows a similar *in silico* risk as BeneFIX at R318Y

Nasdaq: CBIO

In Silico immunogenicity assessment at the R318Y site

	Frame Start	AA Sequence	Frame Stop	Hydro-phobicity	DRB1*0101 Z-Score	DRB1*0301 Z-Score	DRB1*0401 Z-Score	DRB1*0701 Z-Score	DRB1*0801 Z-Score	DRB1*1101 Z-Score	DRB1*1301 Z-Score	DRB1*1501 Z-Score	Hits
BeneFIX	310	WGRVFHKGR	318	-1.2	0.99	0.51	0.61	1.26	1.63	1.39	0.92	0.67	0
	311	GRVFHKGRS	319	-1.19	-0.35	0.22	-0.25	-1.09	1.00	1.04	0.36	1.24	0
	312	RVFHKGRSA	320	-0.94	0.55	0.03	0.50	0.85	-0.87	0.18	1.09	0.32	0
	313	VFHKGRSAL	321	-0.02	0.74	1.71	-0.63	1.41	1.67	1.00	2.03	0.79	3
	314	FHKGRSALV	322	-0.02	2.73	2.29	2.67	2.59	1.74	2.32	2.01	2.48	8
	315	HKGRSALVL	323	0.09	1.24	-0.06	0.30	1.33	0.69	0.54	1.15	1.12	0
	316	KGRSALVLQ	324	0.06	-0.20	0.84	0.26	0.21	0.35	0.47	0.49	0.29	0
	317	GRSALVLQY	325	0.34	0.37	1.27	0.87	0.23	-0.34	0.14	1.38	0.16	0
	318	RSALVLQYL	326	0.81	0.04	0.72	-0.64	0.17	0.26	0.07	-0.26	1.16	0
DalcA	310	WGRVFHKGY	318	-0.84	0.93	0.59	0.53	1.02	0.58	1.31	1.00	0.61	0
	311	GRVFHKGYS	319	-0.83	-0.54	0.03	-0.43	-1.28	0.80	0.84	0.17	1.05	0
	312	RVFHKGYSA	320	-0.59	0.37	-0.11	0.71	0.88	-0.67	-0.12	1.28	0.80	0
	313	VFHKGYSAL	321	0.33	0.76	0.59	-0.63	1.42	0.51	-0.13	0.93	0.59	0
	314	FHKGYSALV	322	0.33	2.58	2.13	2.52	2.44	1.58	2.16	1.85	2.33	7
	315	HKGYSALVL	323	0.44	0.61	-0.06	0.47	1.36	0.72	0.37	1.41	1.49	0
	316	KGYSALVLQ	324	0.41	-0.49	0.55	-0.02	-0.07	0.04	0.18	0.20	0.01	0
	317	GYSALVLQY	325	0.7	0.01	0.90	0.52	-0.13	-0.72	-0.24	1.02	-0.19	0
	318	YSALVLQYL	326	1.17	1.45	1.30	0.73	1.56	1.74	1.51	0.31	1.72	2

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DalcA shows a similar risk as BeneFIX at R338E

Nasdaq: CBIO

In Silico immunogenicity assessment at the R338E site

	Frame Start	AA Sequence	Frame Stop	Hydro-phobicity	DRB1*0101 Z-Score	DRB1*0301 Z-Score	DRB1*0401 Z-Score	DRB1*0701 Z-Score	DRB1*0801 Z-Score	DRB1*1101 Z-Score	DRB1*1301 Z-Score	DRB1*1501 Z-Score	Hits
BeneFIX	330	LVDRATCLR	338	0.32	0.41	-0.46	-0.64	0.26	0.16	0.15	0.50	-0.07	0
	331	VDRATCLRS	339	-0.19	0.47	1.26	1.99	0.42	0.71	0.98	1.37	1.13	1
	332	DRATCLRST	340	-0.73	-0.11	-0.93	-0.47	0.32	0.14	0.34	-0.56	0.40	0
	333	RATCLRSTK	341	-0.78	-1.52	-0.63	-1.28	-1.76	0.34	-0.96	-0.57	-0.92	0
	334	ATCLRSTKF	342	0.03	0.71	0.43	0.91	1.02	0.49	-0.03	0.65	0.43	0
	335	TCLRSTKFT	343	-0.24	0.46	-2.06	-0.18	0.54	0.27	-0.06	-0.74	-0.32	0
	336	CLRSTKFTI	344	0.33	0.67	1.45	-1.15	0.53	0.26	0.98	1.03	1.19	0
	337	LRSTKFTIY	345	-0.09	0.58	1.21	0.37	0.78	0.23	-0.51	2.00	0.59	1
	338	RSTKFTIYN	346	-0.9	-0.90	-0.66	-0.70	-0.56	0.26	-0.75	-0.22	-0.73	0
DalcA	330	LVDRATCLE	338	0.43	-0.21	-0.86	-0.99	0.03	0.31	-0.22	0.10	-0.66	0
	331	VDRATCLES	339	-0.08	0.23	1.02	1.76	0.18	0.46	0.74	1.13	0.90	1
	332	DRATCLEST	340	-0.62	-0.43	-1.01	-0.51	0.17	0.11	-0.38	-0.64	0.13	0
	333	RATCLESTK	341	-0.67	-2.31	-1.39	-1.28	-1.89	-0.45	-1.72	-1.32	-1.49	0
	334	ATCLESTKF	342	0.14	0.43	0.15	0.64	0.74	0.20	-0.32	0.37	0.16	0
	335	TCLESTKFT	343	-0.13	0.78	-1.21	0.84	0.65	-0.77	-0.01	-1.18	-0.94	0
	336	CLESTKFTI	344	0.44	0.21	0.99	-1.59	0.08	-0.22	0.52	0.58	0.76	0
	337	LESTKFTIY	345	0.02	0.07	0.69	-0.13	0.27	-0.31	-1.04	1.49	0.09	0
	338	ESTKFTIYN	346	-0.79	-0.82	-1.30	-0.62	-0.48	0.34	-0.67	-0.85	-1.34	0

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DalcA shows a similar risk as BeneFIX at T343R

Nasdaq: CBIO

In Silico immunogenicity assessment at the T343R site

BeneFIX

Frame Start	AA Sequence	Frame Stop	Hydro-phobicity	DRB1*0101 Z-Score	DRB1*0301 Z-Score	DRB1*0401 Z-Score	DRB1*0701 Z-Score	DRB1*0801 Z-Score	DRB1*1101 Z-Score	DRB1*1301 Z-Score	DRB1*1501 Z-Score	Hits
335	TCLRSTKFT	343	-0.24	0.46	-2.06	-0.18	0.54	0.27	-0.06	-0.74	-0.32	0
336	CLRSTKFTI	344	0.33	0.67	1.45	-1.15	0.53	0.26	0.98	1.03	1.19	0
337	LRSTKFTIY	345	-0.09	0.58	1.21	0.37	0.78	0.23	-0.51	2.00	0.59	1
338	RSTKFTIYN	346	-0.9	-0.90	-0.66	-0.70	-0.56	0.26	-0.75	-0.22	-0.73	0
339	STKFTIYNN	347	-0.79	-0.98	-1.59	-0.09	-0.20	0.10	-0.18	-0.48	0.53	0
340	TKFTIYNNM	348	-0.49	0.53	-0.35	-0.47	1.00	-0.97	-0.84	-0.31	0.31	0
341	KFTIYNNMF	349	-0.1	0.50	0.57	1.19	1.15	-0.03	0.95	0.13	0.55	0
342	FTIYNNMFC	350	0.61	1.21	0.24	1.37	1.14	2.25	1.44	0.62	2.53	2
343	TIYNNMFCA	351	0.5	-0.19	-0.66	-0.87	-0.72	-0.82	0.20	-0.69	-0.40	0

DalcA

335	TCLRSTKFR	343	-0.67	0.10	-0.91	-0.47	-0.12	0.64	-0.38	0.39	-0.67	0
336	CLRSTKFRI	344	-0.09	0.80	1.58	-1.02	0.66	0.40	1.12	1.16	1.32	0
337	LRSTKFRIY	345	-0.51	0.72	1.28	0.34	0.98	0.35	0.80	1.61	0.50	0
338	RSTKFRIYN	346	-1.32	-1.16	-0.40	-2.08	-0.83	0.53	-0.49	0.03	-0.70	0
339	STKFRIYNN	347	-1.21	-0.76	-1.38	0.12	0.01	0.33	0.04	-0.26	0.74	0
340	TKFRIYNNM	348	-0.91	0.52	-0.48	-1.03	0.52	-0.69	-0.44	-0.18	0.19	0
341	KFRIYNNMF	349	-0.52	0.98	1.05	1.66	1.62	0.47	1.44	0.61	1.01	1
342	FRIYNNMFC	350	0.19	1.46	0.51	1.62	1.39	2.52	1.70	0.88	2.78	3
343	RIYNNMFCA	351	0.08	-0.30	-0.02	-0.97	-0.82	-0.93	0.09	-0.07	0.21	0

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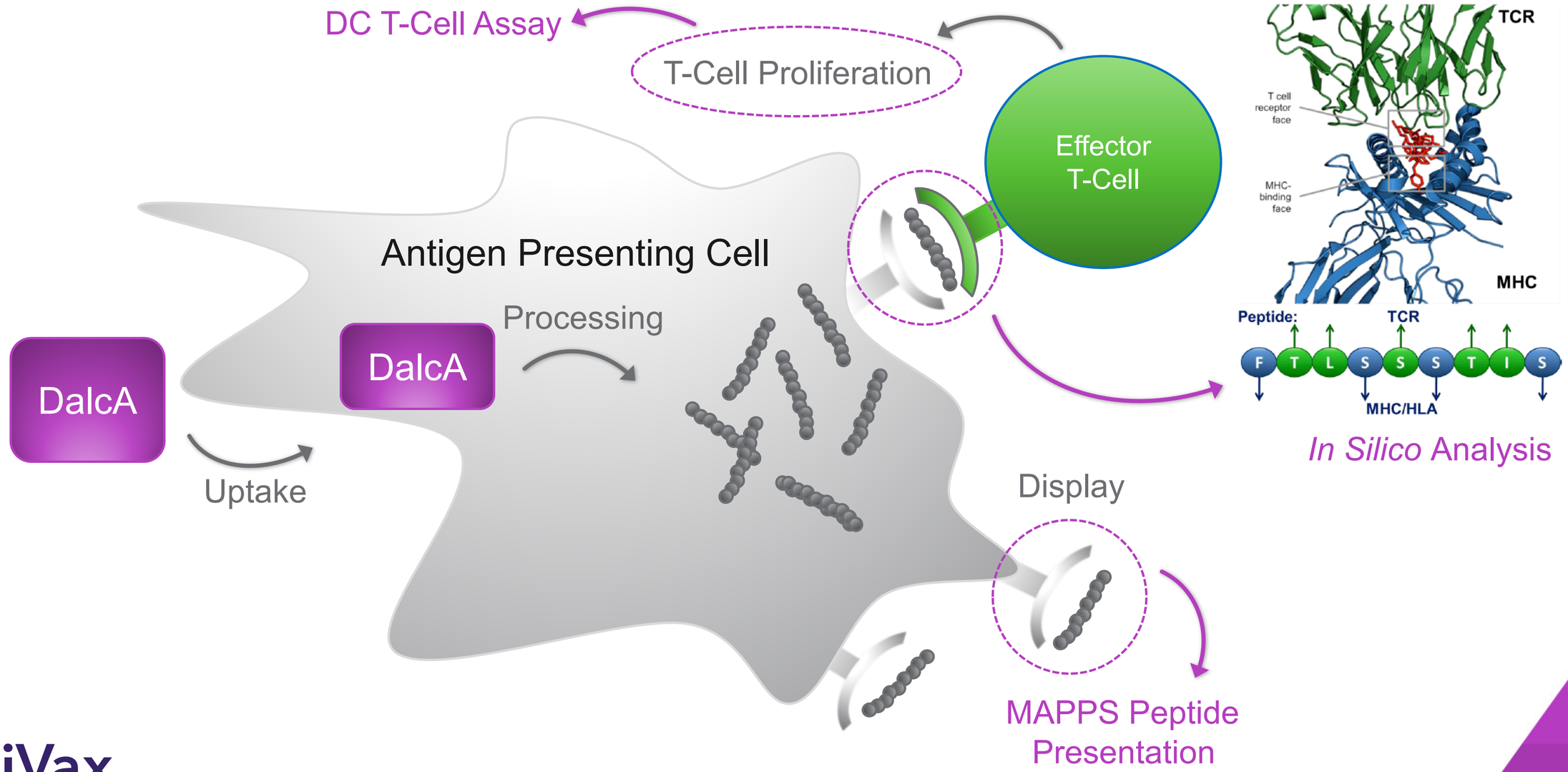
Preclinical toolkit for evaluation of immunogenicity



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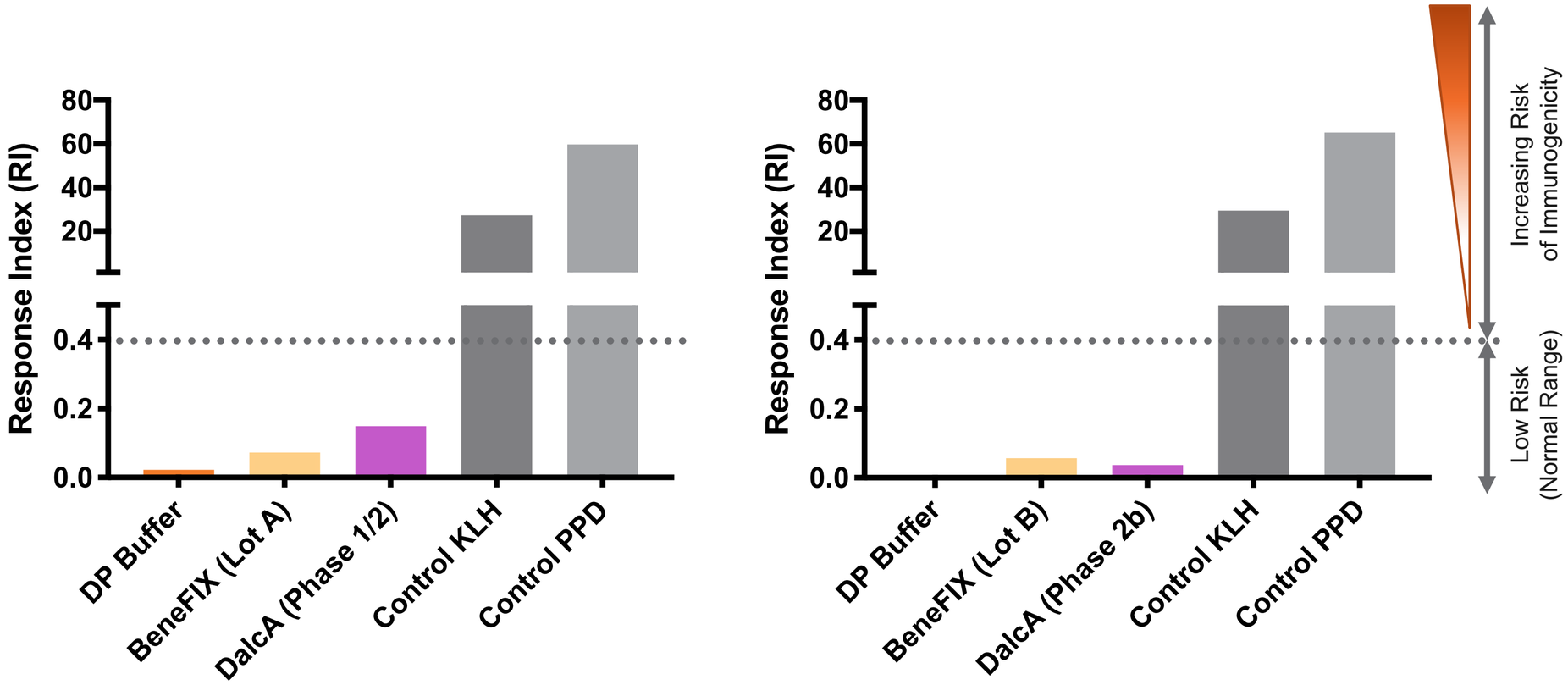
PROIMMUNE www.proimmune.com

DalcA drug product shows low immunogenicity risk

Nasdaq: CBIO

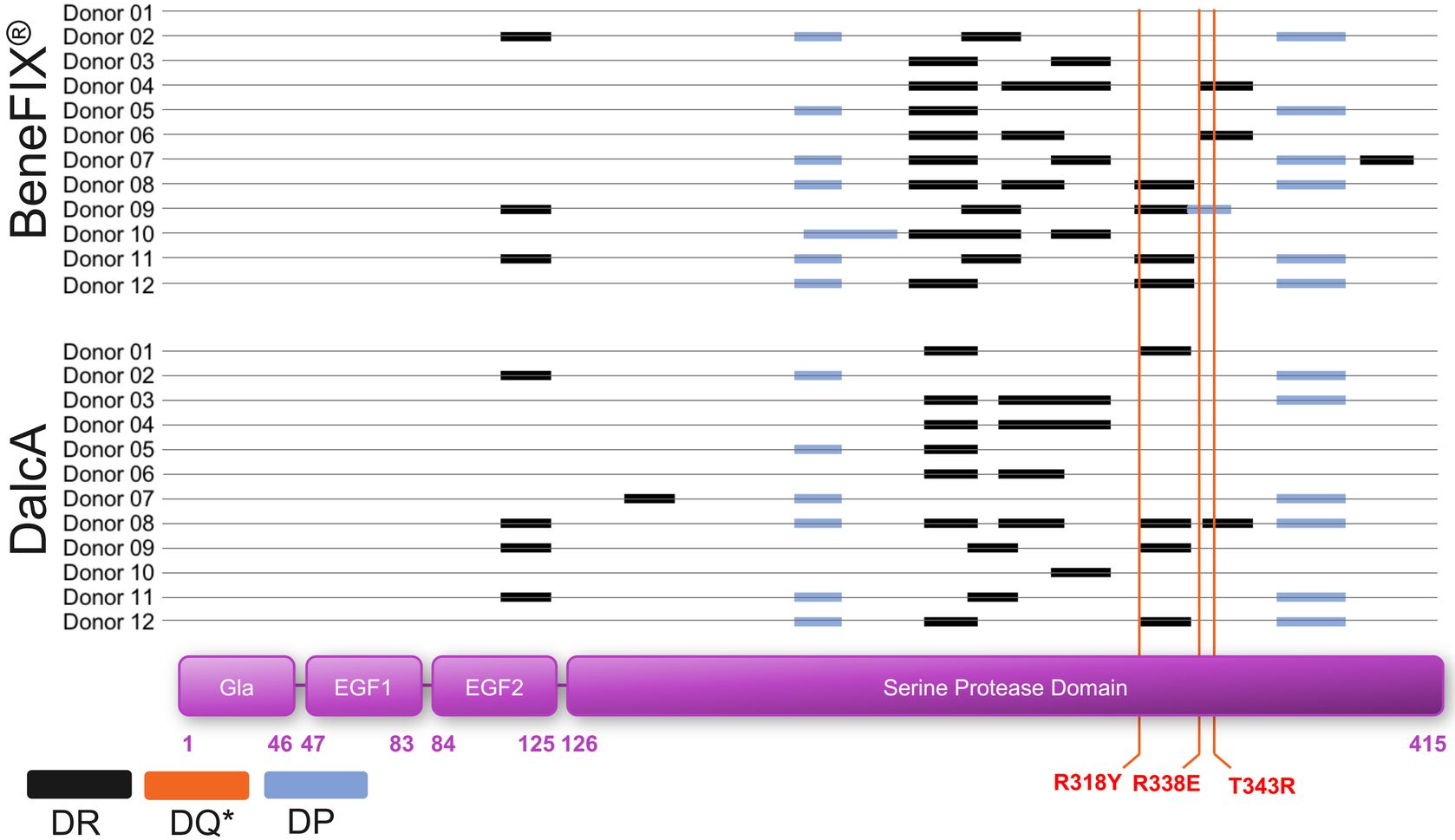
Clinical therapeutics with low risk have Response Index values (RI) between 0.1 and 0.4



+ Dendritic cell T-cell responses to DalcA and BeneFIX were comparable, showing a low response and frequency of stimulation (ProScern - ProImmune)
+ Overall immunogenicity risk profile risk is low and on par with BeneFIX

MAPPS shows comparability for DalcA and BeneFIX

Nasdaq: CBIO



- A major histocompatibility complex (“MHC”)-associated peptide proteomics (“MAPPS”) assay directly identified peptides presented by antigen-presenting cells when loaded with DalcA or BeneFIX (ProPresent - ProImmune)
- Only a single peptide in 1/12 donors was identified for HLA-DQ (173–186 region)

Strong correlation between *in silico* and MAPPS data

T-cell epitope clusters identified by *in silico* screening were presented in MAPPS assays

Input Sequence	Cluster Address	Cluster Sequence	Hydro-phobicity	EpiMatrix Hits	EpiMatrix Cluster Score	JanusMatrix Human Homology	# Donors Presenting WT FIX	# Donors Presenting DalcA
FIX (WT)	112 - 126	TEGYRLAENQKSCEP	-1.51	7	17.74	4.43	3	4
FIX (WT)	191 - 207	QFPWQVVLNGKVDAFCG	0.3	7	12.47	1.00	0	0
FIX (WT)	256 - 277	HHNYNAAINKYNHDIALLELDE	-0.83	9	12.64	1.22	4	2
FIX (WT)	296 - 311	TNIFLKFGSGYVSGWG	0.29	6	11.04	1.29	4	3
FIX (WT)	311 - 334	GRVFHKGRSALVLQYLRVPLVDRA	0.08	19	33.49	2.09	4	4
JanusMatrix Human Homology >3: Elevated degree of cross-conservation with epitopes derived from human proteome.								
FIX (WT)	310 - 326	WGRVFHKGRSALVLQYL	0.06	11	16.82	1.73	4	4
DalcA	310 - 326	WGRVFHKG**Y**SALVLQYL	0.25	9	12.03	0.67		
FIX (WT)	330 - 351	LVDRATCLRSTKFTIYNNMFCA	0.22	4	-2.77	1.5	2	1
DalcA	330 - 351	LVDRATCL**E**STKF**R**IYNNMFCA	0.09	5	-1.12	0.20		

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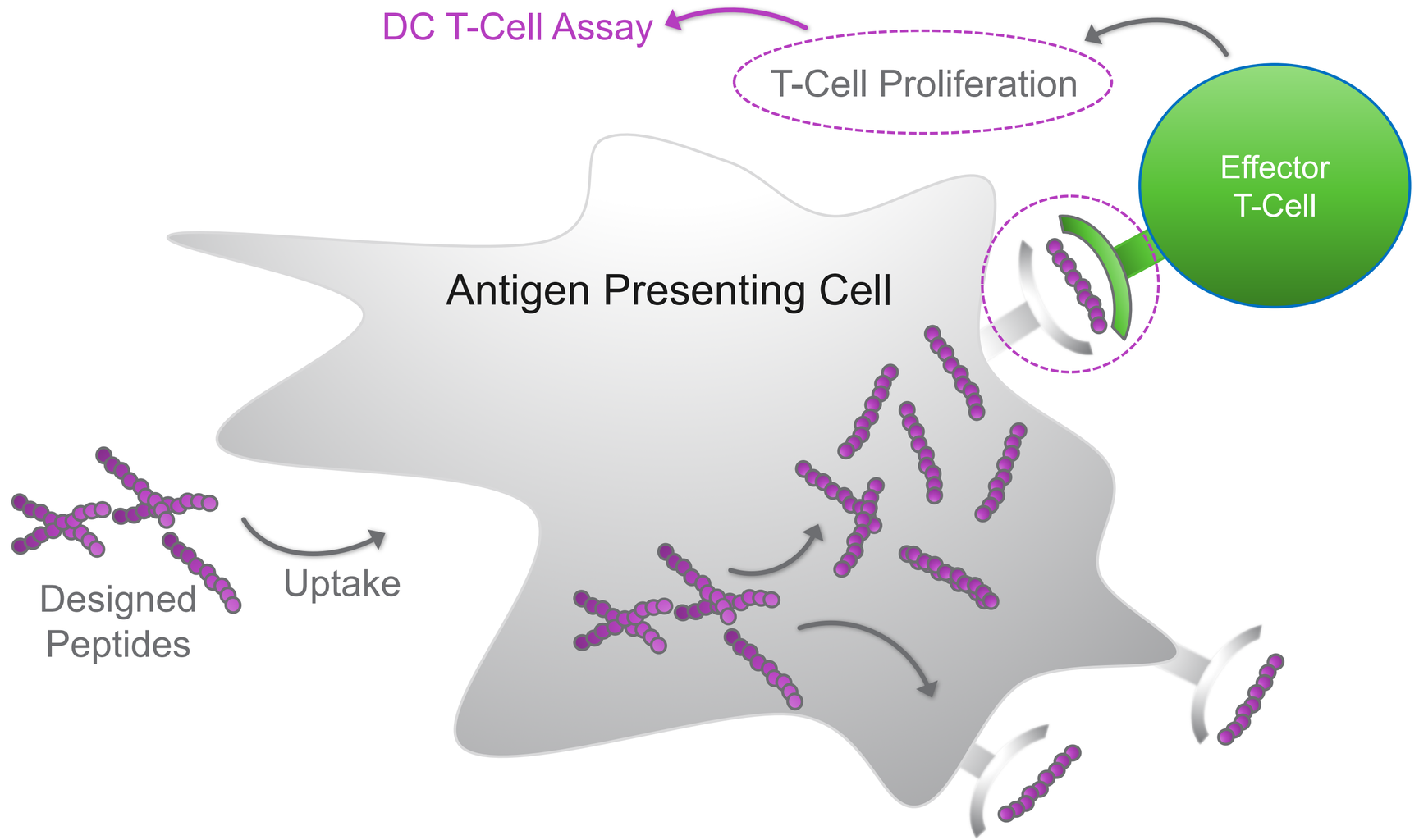
+ 4/5 T-cell epitope clusters identified by *in silico* screening in wild-type FIX were presented by DalcA and BeneFIX in the MAPPS assays (top panel) with some overlap to peptides containing substituted residues (lower panel)

Preclinical toolkit for evaluation of immunogenicity



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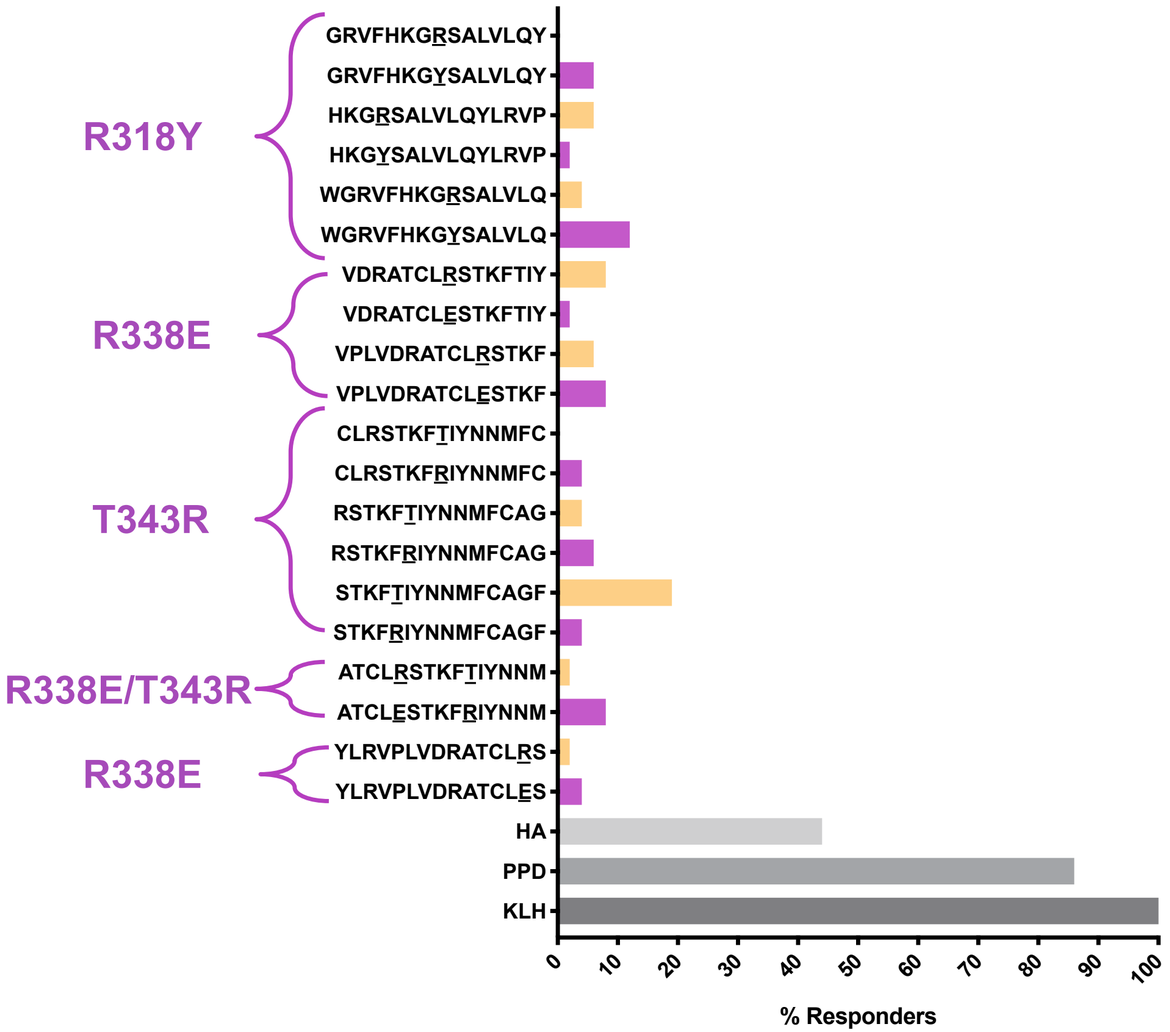
Peptides from DalcA show low immunogenicity risk

% Responding Donors



DC-T cell stimulation: Peptides

+ Overall immunogenicity risk profile for the individual peptides is low and on par with BeneFIX
+ Peptides covered all three amino acid substitutions and selected from *in silico* data
+ Peptides identified in MAPPS experiment have partial or full overlap with tested peptides

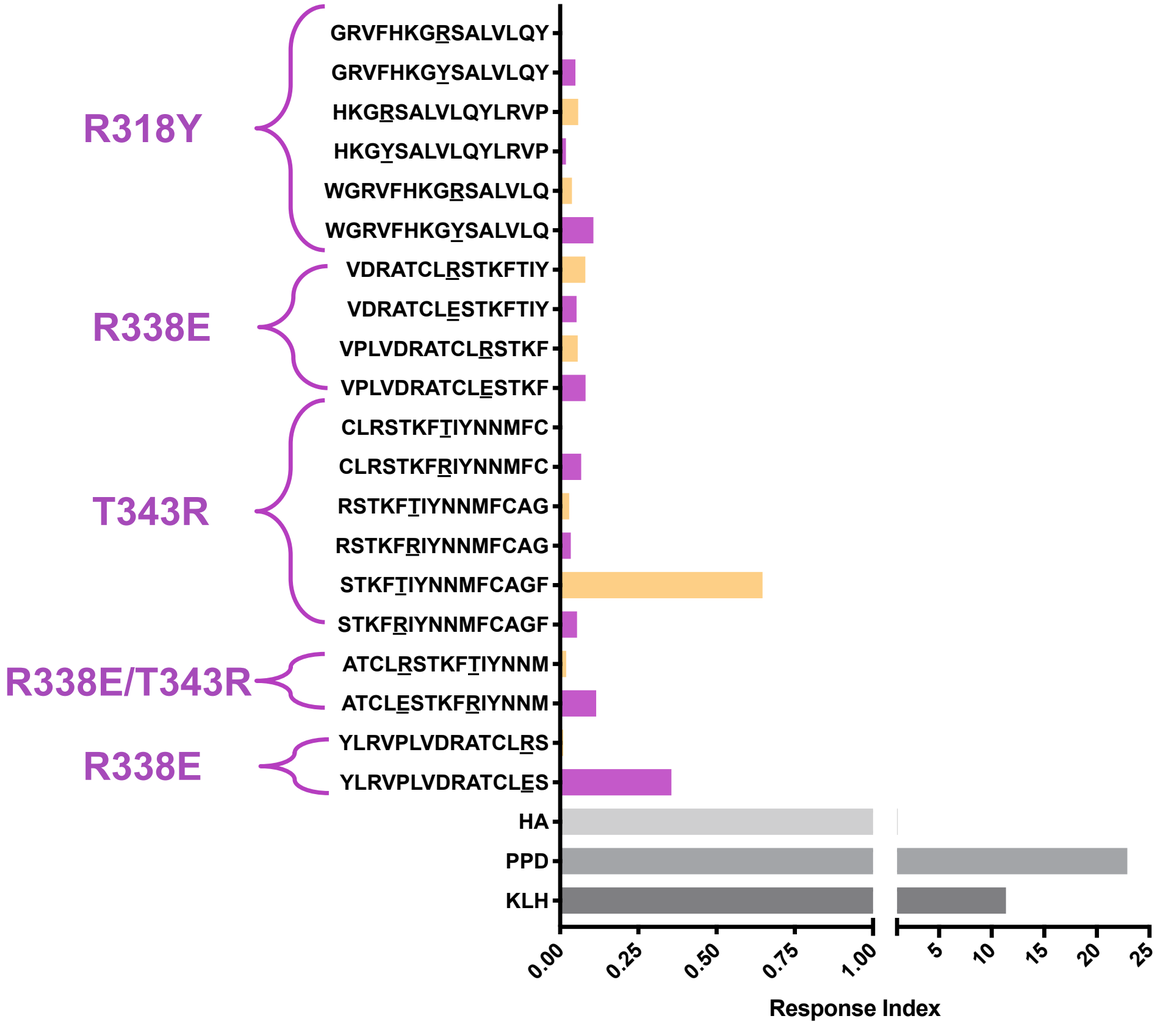
BeneFIX derived peptide

DalcA derived peptide

Control peptide or protein

Peptides from DalcA show low immunogenicity risk

Response Index



DC-T cell stimulation: Peptides

+ Overall immunogenicity risk profile for the individual peptides is low and on par with BeneFIX
+ Peptides covered all three amino acid substitutions and selected from *in silico* data
+ Peptides identified in MAPPS experiment have partial or full overlap with tested peptides

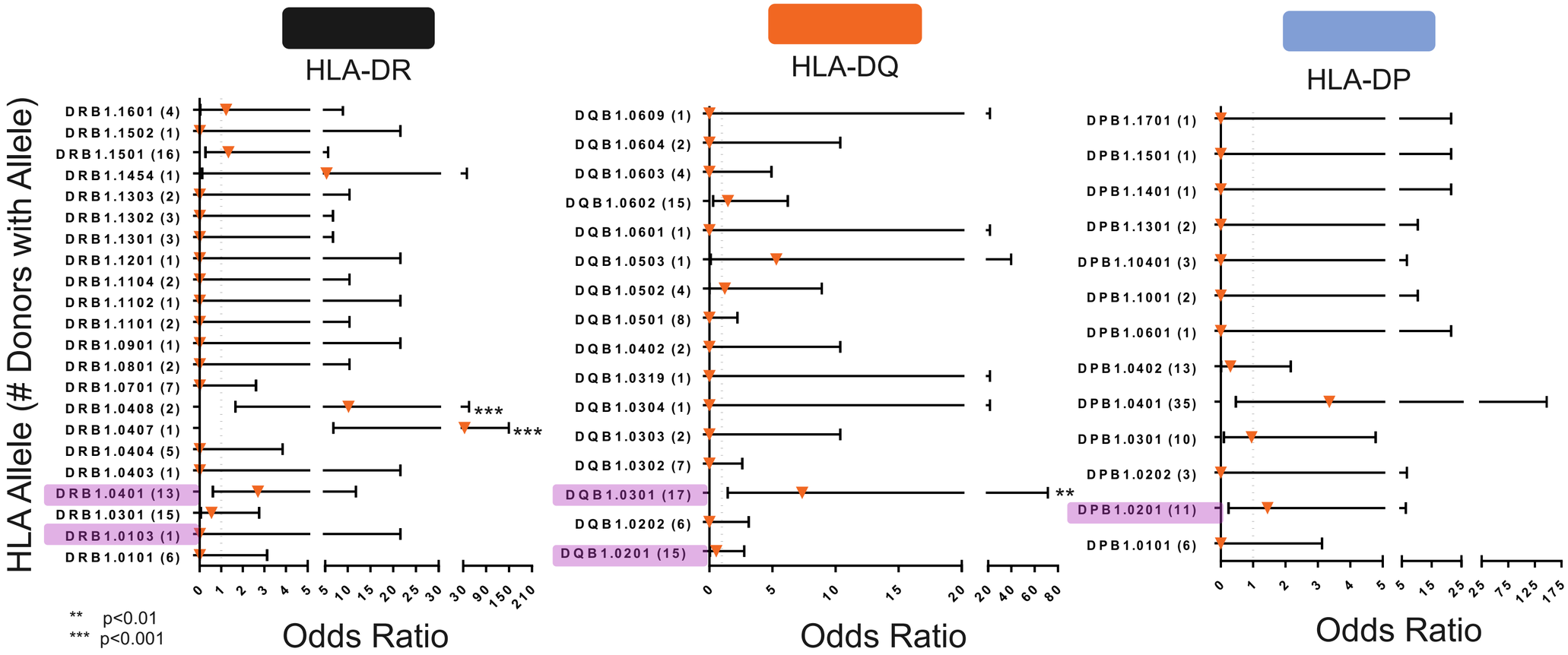
BeneFIX derived peptide

DalcA derived peptide

Control peptide or protein

Correlation of HLA status & T-cell response

Nasdaq: CBIO



Alleles expressed by patient who developed high titer nAb response

- Positive responses were defined as proliferation (> 1 S.D.) exceeding control
- Only HLA alleles DRB1*04:07, DRB1*04:08 and DQB1*03:01 were significantly associated with an increased odds of positive response (Odds Ratio >1)

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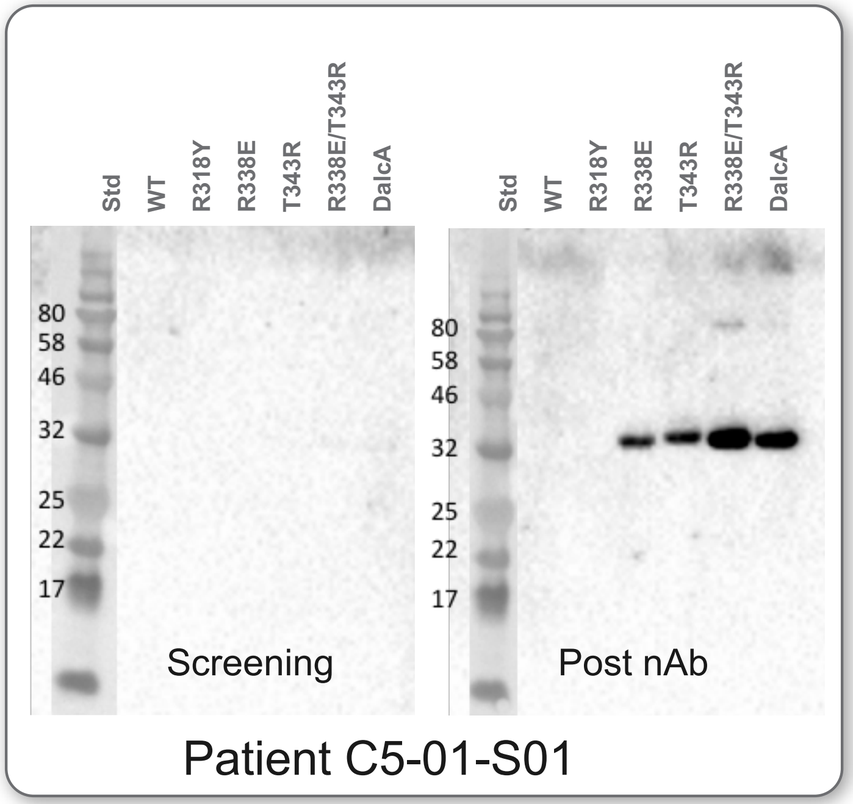
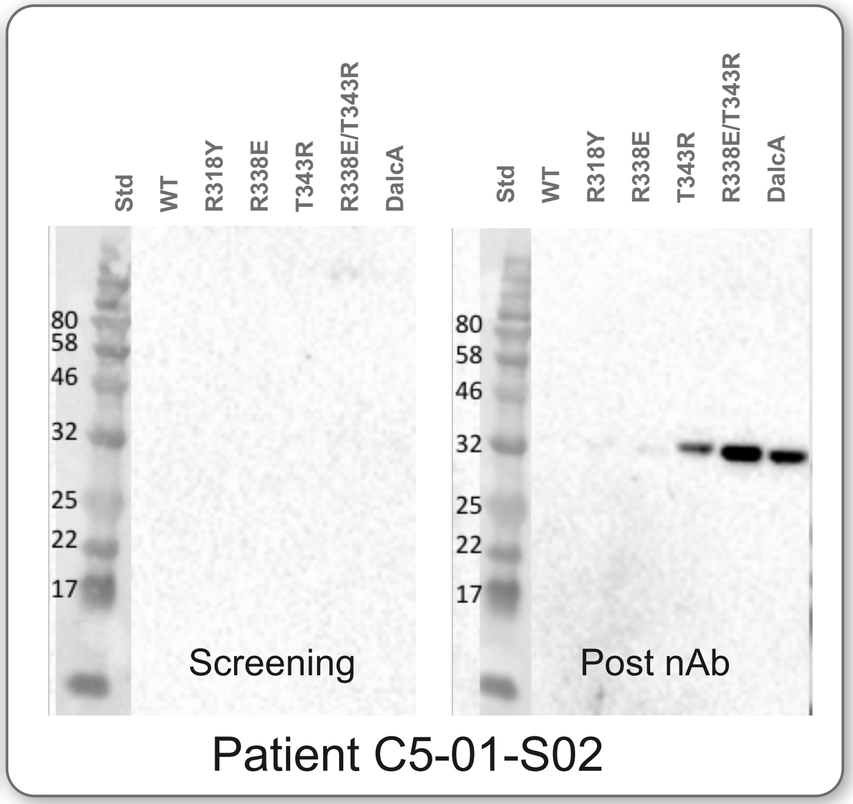
B-cell epitope mapping identified the T343R region

Nasdaq: CBIO

B-cell epitope mapping using single site variants of DalcA identified the binding region





- B-cell epitope mapping identified the R338E/T343R region to be targeted by neutralizing antibodies in both subjects
- Confirmed the absence of cross-reactivity to WT FIX

DalcA is comparable to BeneFIX & RIXUBIS

Multiple industry standard characterizations performed

- Potency
- Biological Activity
- Product Purity
- Biophysical and Structural Properties
- Chemical Modifications
- Post Translational Modifications
- Host Cell Impurities
- Product and Process Related Impurities
- Thermal Stability upon Reconstitution

Product quality & stability attributes are comparable to marketed rFIX products

What may have led to the development of nAbs?

The DalcA molecule is not inherently immunogenic – What now to consider in the clinic

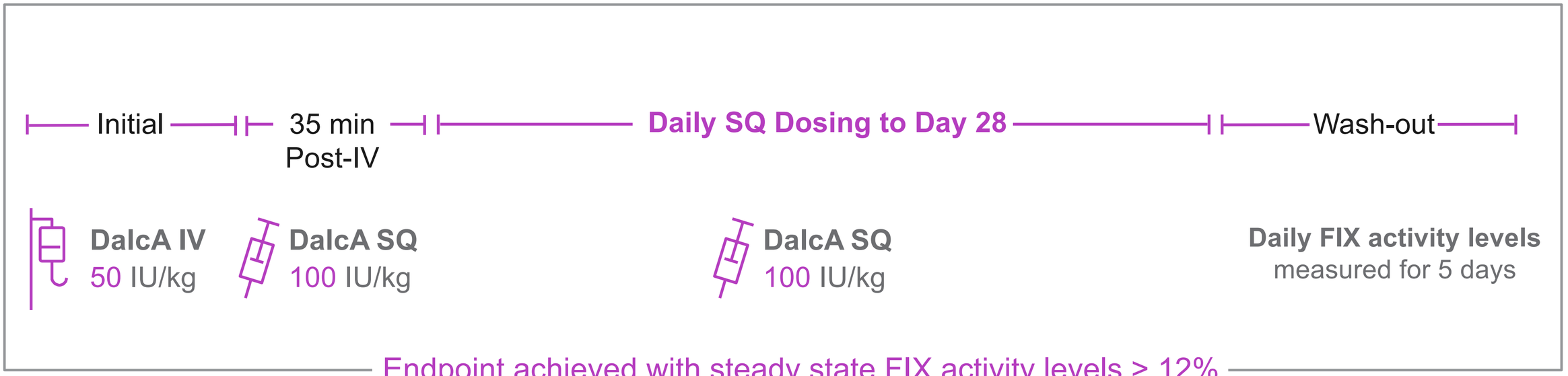
- The nAbs were a rare event observed early in the trial within a restricted population
- The nAbs were associated with the rare genotype and/or certain HLA types
- The nAbs did not cross-react with BeneFIX or RIXUBIS so do not present a safety risk

Conclusion – Evaluate further safety & efficacy in a Phase 2b trial

+ Broaden the subject population to have a diverse ethnic and genotypic background
+ Exclude the rare genotype of the two subjects who developed nAbs in the P1/2 trial
+ Consider HLA profile and exclude those with HLA types that may be deemed at risk
+ Execute the P2b trial (28 days of dosing) with careful monitoring for development of nAbs

DalcA Phase 2b SQ clinical trial design: DLZ-201

Ongoing and currently enrolling the phase 2b study: DLZ-201



- Enrollment: 6 patients
- Single IV dose followed by 28 day SQ dosing
- Primary endpoint: Steady state FIX activity level above 12% with daily dosing
- Secondary endpoints: no inhibitor formation, pharmacokinetics, pharmacodynamics

Conclusions on the dalcinonacog alfa program

Clinical development after an extensive immunogenicity risk assessment

Preclinical immunogenicity assessment showed that dalcinonacog alfa is equivalent to that of competitors such as BeneFIX

A comprehensive evaluation of the drug product showed comparable quality to marketed rFIX products

KoLs and subject experts agree with the immunogenicity risk assessment and proceeding with the P2b to evaluate the safety and efficacy of dalcinonacog alfa

THANK YOU

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